

We claim:

1. Isolated DNA sequence coding for a tumor rejection antigen.
2. Isolated DNA sequence of claim 1, coding for a tumor rejection antigen specific for mastocytoma.
3. Isolated DNA sequence of claim 1, coding for tumor rejection antigen P815.
4. Isolated DNA sequence of claim 1, comprising three exons having the nucleotide sequence of figure 6 and sequence of id no: 1.
5. Isolated DNA sequence of claim 1, coding for a human tumor rejection antigen.
6. Isolated DNA sequence of claim 5, wherein said tumor rejection antigen is characteristic of melanoma.

7. Isolated DNA sequence of claim 6, wherein said tumor rejection antigen is melanoma antigen E, and has nucleic acid sequence:

	10	20	30	40	50	60
1	GGATCCAGGC	CCTGCCAGGA	AAAATATAAG	GGCCCTGCGT	GAGAACAGAG	GGGGTCATCC 60
61	ACTGCATGAG	AGTGGGGATG	TCACAGAGTC	CAGCCCACCC	TCCTGGTAGC	ACTGAGAAGC 120
121	CAGGGCTGTG	CTTGGCGTCT	GCACCCTGAG	GGCCCGTGGG	TTCTCTTCC	TGGAGCTCCA 180
181	GGAACCAGGC	AGTGAGGCCT	TGGTCTGAGA	CAGTATCCTC	AGGTCACAGA	GCAGAGGATG 240
241	CACAGGGTGT	GCCAGCAGTG	AATGTTTGCC	CTGAATGCAC	ACCAAGGGCC	CCACCTGCCA 300
301	CAGGACACAT	AGGACTCCAC	AGAGTCTGGC	CTCACCTCCC	TACTGTCACT	CCTGTAGAAT 360
361	CGACCTCTGC	TGGCCGGCTG	TACCCTGAGT	ACCCTCTCAC	TTCTCTTTC	AGGTTTTTCAG 420
421	GGGACAGGCC	AACCCAGAGG	ACAGGATTCC	CTGGAGGCCA	CAGAGGAGCA	CCAAGGAGAA 480
481	GATCTGTAAG	TAGGCCTTTG	TTAGAGTCTC	CAAGGTTTCAG	TTCTCAGCTG	AGGCCTCTCA 540
541	CACACTCCCT	CTCTCCCCAG	GCCTGTGGGT	CTTCATTGCC	CAGCTCCTGC	CCACACTCCT 600
601	GCCTGCTGCC	CTGACGAGAG	TCATCATGTC	TCTTGAGCAG	AGGAGTCTGC	ACTGCAAGCC 660
661	TGAGGAAGCC	CTTGAGGCC	AACAAGAGGC	CCTGGGCTGG	TGTGTGTGCA	GGCTGCCACC 720
721	TCCTCCTCCT	CTCCTCTGGT	CCTGGGCACC	CTGGAGGAGG	TGCCCCACTGC	TGGGTCAACA 780
781	GATCCTCCCC	AGAGTCCTCA	GGGAGCCTCC	GCCTTTCCCA	CTACCATCAA	CTTCACTCGA 840
841	CAGAGGCAAC	CCAGTGAGGG	TTCCAGCAGC	CGTGAAGAGG	AGGGGCCAAG	CACCTCTTGT 900
901	ATCCTGGAGT	CCTTGTTCCG	AGCAGTAATC	ACTAAGAAGG	TGGCTGATTT	GGTTGGTTTT 960
961	CTGCTCCTCA	AATATCGAGC	CAGGGAGCCA	GTCACAAAGG	CAGAAATGCT	GGAGAGTGTC 1020
1021	ATCAAAAATT	ACAAGCACTG	TTTTCTTGAG	ATCTTCGGCA	AAGCCTCTGA	GTCTTGCGAG 1080
1081	CTGGTCTTTG	GCATTGACGT	GAAGGAAGCA	GACCCACCCG	GCCACTCCTA	TGTCCTTGTC 1140
1141	ACCTGCCTAG	GTCTCTCCTA	TGATGGCCTG	CTGGGTGATA	ATCAGATCAT	GCCCAAGACA 1200
1201	GGCTTCCTGA	TAATTGTCCT	GGTCATGATT	GCAATGGAGG	GCGGCCATGC	TCCTGAGGAG 1260
1261	GAAATCTGGG	AGGAGCTGAG	TGTGATGGAG	GTGTATGATG	GGAGGGAGCA	CAGTGCCAT 1320
1321	GGGGAGCCCA	GGAAGCTGCT	CACCCAAGAT	TTGGTGCAGG	AAAAGTACCT	GGAGTACGGC 1380
1381	AGGTGCCGGA	CAGTGATCCC	GCACGCTATG	AGTTCTCTGT	GGGTCCAAGG	GCCCTCGCTG 1440
1441	AAACCAGCTA	TGTGAAAGTC	CTTGAGTATG	TGATCAAGGT	CAGTGCAAGA	GTTCGCTTTT 1500
1501	TCTTCCCATC	CCTGCGTGAA	GCAGCTTTGA	GAGAGGAGGA	AGAGGGAGTC	TGAGCATGAG 1560
1561	TTGCAGCCAA	GGCCAGTGGG	AGGGGGACTG	GGCCAGTGCA	CCTTCCAGGG	CCGCGTCCAG 1620
1621	CAGCTTCCCC	TGCCTCGTGT	GACATGAGGC	CCATTCTTCA	CTCTGAAGAG	AGCGGTCACT 1680
1681	GTTCTCAGTA	GTAGGTTTCT	GTTCTATTGG	GTGACTTGGA	GATTATCTTT	TGTTCTCTTT 1740
1741	TGGAATTGTT	CAAATGTTTT	TTTTTAAGGG	ATGGTTGAAT	GAACCTCAGC	ATCCAAGTTT 1800
1801	ATGAATGACA	GCAGTCACAC	AGTTCTGTGT	ATATAGTTTA	AGGGTAAGAG	TCTTGTGTTT 1860
1861	TATTCAGATT	GGGAAATCCA	TTCTATTTTG	TGAATTGGGA	TAATAACAGC	AGTGGGAATA 1920
1921	GTACTTAGAA	ATGTGAAAAA	TGAGCAGTAA	AATAGATGAG	ATAAAGAACT	AAAGAAATTA 1980
1981	AGAGATAGTC	AATTCTTGCC	TTATACCTCA	GTCTATTCTG	TAAAATTTTT	AAAGATATAT 2040
2041	GCATACCTGG	ATTTCTTGCC	CTTCTTTGAG	AATGTAAGAG	AAATTAATC	TGAATAAAGA 2100
2101	ATTCTTCCTG	TTCACTGGCT	CTTTTCTTCT	CCATGCACTG	AGCATCTGCT	TTTTTGAAGG 2160
2161	CCCTGGGTTA	GTAGTGGAGA	TGCTAAGGTA	AGCCAGACTC	ATACCCACCC	ATAGGGTCGT 2220
2221	AGAGTCTAGG	AGCTGCAGTC	ACGTAATCGA	GGTGGCAAGA	TGTCCTCTAA	AGATGTAGGG 2280
2281	AAAAGTGAGA	GAGGGGTGAG	GGTGTGGGGC	TCCGGGTGAG	AGTGGTGGAG	TGTCAATGCC 2340
2341	CTGAGCTGGG	GCAATTTTGGG	CTTTGGGAAA	CTGCAGTTCC	TTCTGGGGGA	GCTGATTGTA 2400
2401	ATGATCTTGG	GTGGATCC				2418

8. Biologically pure culture of a cell line transfected with the isolated DNA sequence of claim 1.

9. Biologically pure culture of claim 5, wherein said cell line is selected from the group consisting of P1A.T2 and P1A.TC3.1.

10. Biologically pure culture of a highly transfectable cell line derived from a parent cell line which expresses at least one P815 tumor antigen, wherein said highly transfectable cell line does not express any of P815 tumor antigens A, B and C.

11. Biologically pure cell line of claim 7, comprising cell line PO.HTR.

12. Biologically pure cell line of claim 7, wherein said isolated DNA sequence is a human tumor rejection antigen.

13. Biologically pure cell line of claim 12, wherein said tumor rejection antigen is characteristic of melanoma.

14. Biologically pure cell line of claim 13, said tumor rejection antigen is melanoma antigen E and isolated DNA has nucleic acid sequence:

	10	20	30	40	50	60	
1	GGATCCAGGC	CCTGCCAGGA	AAAATATAAG	GGCCCTGCGT	GAGAACAGAG	GGGGTCATCC	60
61	ACTGCATGAG	AGTGGGGATG	TCACAGAGTC	CAGCCCACCC	TCCTGGTAGC	ACTGAGAAGC	120
121	CAGGGCTGTG	CTTGCGGTCT	GCACCCTGAG	GGCCCGTGGA	TTCTCTTCC	TGGAGCTCCA	180
181	GGAACCAAGC	AGTGAGGCCT	TGGTCTGAGA	CAGTATCCTC	AGGTACACAG	GCAGAGGATG	240
241	CACAGGGTGT	GCCAGCAGTG	AATGTTTGCC	CTGAATGCAC	ACCAAGGGCC	CCACCTGCCA	300
301	CAGGACACAT	AGGACTCCAC	AGAGTCTGGC	CTCACCTCCC	TACTGTCACT	CCTGTAGAAT	360
361	CGACCTCTGC	TGGCCGGCTG	TACCTGAGT	ACCTCTCAC	TTCTCTTTC	AGGTTTTCAG	420
421	GGGACAGGCC	AACCCAGAGG	ACAGGATTCC	CTGGAGGCCA	CAGAGGAGCA	CCAAGGAGAA	480
481	GATCTGTAAG	TAGGCCTTTG	TTAGAGTCTC	CAAGGTTTCA	TTCTCAGCTG	AGGCCTCTCA	540
541	CACACTCCCT	CTCTCCCCAG	GCCTGTGGGT	ETTCATTGCC	CAGCTCCTGC	CCCACTCCT	600
601	GCCTGCTGCC	CTGACGAGAG	TCATCATGTC	TCTTGAGCAG	AGGAGTCTGC	ACTGCAAGCC	660
661	TGAGGAAGCC	CTTGAGGCC	AACAAGAGGC	CCTGGGCTGG	TGTGTGTGCA	GGCTGCCACC	720
721	TCCTCTCCT	CTCCTCTGGT	CCTGGGCACC	CTGGAGGAGG	TGCCCACTGC	TGGGTCAACA	780
781	GATCCTCCCC	AGAGTCCTCA	GGGAGCCTCC	GCCTTTCCCA	CTACCATCAA	CTTCACTCGA	840
841	CAGAGGCAAC	CCAGTGAGGG	TTCCAGCAGC	CGTGAAGAGG	AGGGGCCAAG	CACCTCTTGT	900
901	ATCCTGGAGT	CTTGTTCGG	AGCAGTAATC	ACTAAGAAGG	TGGCTGATTT	GGTTGGTTTT	960
961	CTGCTCCTCA	AATATCGAGC	CAGGGAGCCA	GTCACAAAGG	CAGAAATGCT	GGAGAGTGTC	1020
1021	ATCAAAATT	ACAAGCACTG	TTTTCTGAG	ATCTTCGGCA	AAGCCTCTGA	GTCTTGCGAG	1080
1081	CTGGTCTTTG	GCATTGACGT	GAAGGAAGCA	GACCCACCG	GCCACTCCTA	TGTCCTTGTC	1140
1141	ACCTGCCTAG	GTCTCTCCTA	TGATGGCCTG	CTGGGTGATA	ATCAGATCAT	GCCCAAGACA	1200
1201	GGCTTCCTGA	TAATTGTCCT	GGTCATGATT	GCAATGGAGG	GCGGCCATGC	TCCTGAGGAG	1260
1261	GAAATCTGGG	AGGAGCTGAG	TGTGATGGAG	GTGTATGATG	GGAGGGAGCA	CAGTGCCCTAT	1320
1321	GGGGAGCCCA	GGAAGCTGCT	CACCCAAGAT	TTGGTGCAAG	AAAAGTACCT	GGAGTACGGC	1380
1381	AGGTGCCGGA	CAGTGATCCC	GCACGCTATG	AGTTCCTGTG	GGGTCCAAGG	GCCCTCGCTG	1440
1441	AAACCAGCTA	TGTGAAAGTC	CTTGAGTATG	TGATCAAGGT	CAGTGCAAGA	GTTGCTTTT	1500
1501	TCTTCCCATC	CCTGCGTGAA	GCAGCTTTGA	GAGAGGAGGA	AGAGGGAGTC	TGAGCATGAG	1560
1561	TTGCAGCCAA	GGCCAGTGGG	AGGGGGACTG	GGCCAGTGCA	CCTTCCAGGG	CCGCGTCCAG	1620
1621	CAGCTTCCCC	TGCCTCGTGT	GACATGAGGC	CCATTCTTCA	CTCTGAAGAG	AGCGGTCAGT	1680
1681	GTTCTCAGTA	GTAGGTTTCT	GTTCTATTGG	GTGACTTGGA	GATTTATCTT	TGTTCTCTTT	1740
1741	TGGAATTGTT	CAAATGTTTT	TTTTTAAGGG	ATGGTTGAAT	GAACTTCAGC	ATCCAAGTTT	1800
1801	ATGAATGACA	GCAGTCACAC	AGTTCCTGTG	ATATAGTTTA	AGGGTAAGAG	TCTTGTGTTT	1860
1861	TATTCAGATT	GGGAAATCCA	TTCTATTTTG	TGAATTGGGA	TAATAACAGC	AGTGGAAATA	1920
1921	GTACTTAGAA	ATGTGAAAAA	TGAGCAGTAA	AATAGATGAG	ATAAAGAACT	AAAGAAATTA	1980
1981	AGAGATAGTC	AATTCTTGCC	TTATACCTCA	GTCTATTCTG	TAAAATTTTT	AAAGATATAT	2040
2041	GCATACCTGG	ATTTCTTGCG	CTTCTTTGAG	AATGTAAGAG	AAATTAAATC	TGAATAAAGA	2100
2101	ATTCTTCTCG	TTCACTGGCT	CTTTTCTTCT	CCATGCACTG	AGCATCTGCT	TTTTGGAAGG	2160
2161	CCCTGGGTTA	GTAGTGGAGA	TGCTAAGGTA	AGCCAGACTC	ATACCCACCC	ATAGGGTCGT	2220
2221	AGAGTCTAGG	AGCTGCAGTC	ACGTAATCGA	GGTGGCAAGA	TGTCCTCTAA	AGATGTAGGG	2280
2281	AAAAGTGAGA	GAGGGGTGAG	GGTGTGGGGC	TCCGGGTGAG	AGTGGTGGAG	TGTCAATGCC	2340
2341	CTGAGCTGGG	GCATTTTGGG	CTTTGGGAAA	CTGCAGTTCC	TTCTGGGGGA	GCTGATTGTA	2400
2401	ATGATCTTGG	GTGGATCC					2418
	10	20	30	40	50	60	

15. Method for diagnosing presence of a tumor in a patient, comprising contacting a lymphocyte containing sample of said patient to a cell line transfected with a DNA sequence coding for a tumor rejection antigen specific for the tumor to be diagnosed, and determining lysis of said transfected cell line by a cytotoxic T cell line said lymphocyte containing sample as an indication of said tumor.

16. Method for treating a patient with a tumor, comprising:  
 (i) removing a lymphocyte containing sample from said patient,  
 (ii) contacting the lymphocyte containing sample to a cell line transfected with a gene coding for and expressing a gene for a tumor rejection antigen specific for the tumor of said patient, under conditions favoring production of cytotoxic T cells against said tumor rejection antigen, and

(iii) introducing said cytotoxic T cells to said patient in an amount sufficient to lyse cells of said tumor.

17. Isolated tumor rejection antigen.

18. Vaccine useful in treating a patient with a tumor comprising a pure peptide or peptide fragment characteristic of the tumor to be treated which binds with an MHC-I or HLA molecule and stimulates production of cytotoxic T cells specific for cells of said tumor.

19. Isolated tumor rejection antigen of claim 10, having the amino acid sequence of figure 7 and sequence id no: 1.

20. Vaccine of claim 18, wherein said pure peptide has the amino acid sequence of figure 7 and sequence id no: 1.

21. Vaccine of claim 20, wherein said tumor is a melanoma.

22. Vaccine of claim 21, wherein said pure peptide or peptide fragment is coded for by nucleic acid sequence:

	10	20	30	40	50	60	
1	GGATCCAGGC	CCTGCCAGGA	AAAATATAAG	GGCCCTGCGT	GAGAACAGAG	GGGGTCATCC	60
61	ACTGCATGAG	AGTGGGGATG	TCACAGAGTC	CAGCCCCACCC	TCCTGGTAGC	ACTGAGAAGC	120
121	CAGGGCTGTG	CTTGCGGTCT	GCACCCTGAG	GGCCCGTGGA	TTCTCTTCC	TGGAGCTCCA	180
181	GGAACCAGGC	AGTGAGGCCT	TGGTCTGAGA	CAGTATCCTC	AGGTCACAGA	GCAGAGGATG	240
241	CACAGGGTGT	GCCAGCAGTG	AATGTTTGCC	CTGAATGCAC	ACCAAGGGCC	CCACCTGCCA	300
301	CAGGACACAT	AGGACTCCAC	AGAGTCTGGC	CTCACCTCCC	TACTGTCAGT	CCTGTAGAAT	360
361	CGACCTCTGC	TGGCCGGCTG	TACCCTGAGT	ACCCTCTCAC	TTCTCCTTC	AGGTTTTTCAG	420
421	GGGACAGGCC	AACCCAGAGG	ACAGGATTCC	CTGGAGGCCA	CAGAGGAGCA	CCAAGGAGAA	480
481	GATCTGTAAG	TAGGCCTTTG	TTAGAGTCTC	CAAGGTTTCT	TTCTCAGCTG	AGGCCTCTCA	540
541	CACACTCCCT	CTCTCCCCAG	GCCTGTGGGT	ETTCATTGCC	CAGCTCCTGC	CCACACTCCT	600
601	GCCTGCTGCC	CTGACGAGAG	TCATCATGTC	TCTTGAGCAG	AGGAGTCTGC	ACTGCAAGCC	660
661	TGAGGAAGCC	CTTGAGGCC	AACAAGAGGC	CCTGGGCTGG	TGTGTGTGCA	GGCTGCCACC	720
721	TCCTCCTCCT	CTCCTCTGGT	CCTGGGCCACC	CTGGAGGAGG	TGCCCCACTGC	TGGGTCAACA	780
781	GATCCTCCCC	AGAGTCCTCA	GGGAGCCTCC	GCCTTTCCCA	CTACCATCAA	CTTCACTCGA	840
841	CAGAGGCAAC	CCAGTGAGGG	TTCCAGCAGC	CGTGAAGAGG	AGGGGGCCAAG	CACCTCTTGT	900
901	ATCCTGGAGT	CCTTGTTCCG	AGCAGTAATC	ACTAAGAAGG	TGGCTGATTT	GGTTGGTTTT	960
961	CTGCTCCTCA	AATATCGAGC	CAGGGAGCCA	GTCACAAAGG	CAGAAATGCT	GGAGAGTGTC	1020
1021	ATCAAAAATT	ACAAAGCACTG	TTTTCTTGAG	ATCTTCGGCA	AAGCCTCTGA	GTCCTTGAG	1080
1081	CTGGTCTTTG	GCATTGACGT	GAAGGAAGCA	GACCCACCCG	GCCACTCCTA	TGTCCTTGTC	1140
1141	ACCTGCCTAG	GTCTCTCCTA	TGATGGCCTG	CTGGGTGATA	ATCAGATCAT	GCCCCAAGACA	1200
1201	GGCTTCCTGA	TAATTGTCTT	GGTCATGATT	GCAATGGAGG	GCGGCCATGC	TCCTGAGGAG	1260
1261	GAAATCTGGG	AGGAGCTGAG	TGTGATGGAG	GTGTATGATG	GGAGGGAGCA	CAGTGCCTAT	1320
1321	GGGGAGCCCA	GGAAGCTGCT	CACCCAAGAT	TTGGTGCAGG	AAAAGTACCT	GGAGTACGGC	1380
1381	AGGTGCCGGA	CAGTGATCCC	GCACGCTATG	AGTTCTCTGT	GGGTCCAAGG	GCCCTCGCTG	1440
1441	AAACCAGCTA	TGTGAAAGTC	CTTGAGTATG	TGATCAAGGT	CAGTGCAAGA	GTTCCGTTTT	1500
1501	TCTTCCCATC	CCTGCGTGAA	GCAGCTTTGA	GAGAGGAGGA	AGAGGGAGTC	TGAGCATGAG	1560
1561	TTGCAGCCAA	GGCCAGTGGG	AGGGGGACTG	GGCCAGTGCA	CCTTCCAGGG	CCGCGTCCAG	1620
1621	CAGCTTCCCC	TGCCTCGTGT	GACATGAGGC	CCATTCTTCA	CTCTGAAGAG	AGCGGTGAGT	1680
1681	GTTCTCAGTA	GTAGGTTTCT	GTTCTATTGG	GTGACTTGGA	GATTTATCTT	TGTTCTCTTT	1740
1741	TGGAATTGTT	CAAATGTTTT	TTTTTAAGGG	ATGGTTGAAT	GAACTTCAGC	ATCCAAGTTT	1800
1801	ATGAATGACA	GCAGTCACAC	AGTTCTGTGT	ATATAGTTTA	AGGGTAAGAG	TCTTGTGTTT	1860
1861	TATTCAGATT	GGGAAATCCA	TTCTATTTTG	TGAATTGGGA	TAATAACAGC	AGTGGAAATA	1920
1921	GTACTTAGAA	ATGTGAAAAA	TGAGCAGTAA	AATAGATGAG	ATAAAGAACT	AAAGAAATTA	1980
1981	AGAGATAGTC	AATTCTTGCC	TTATACCTCA	GTCTATTCTG	TAAAATTTTT	AAAGATATAT	2040
2041	GCATACCTGG	ATTTCTTTGG	CTTCTTTGAG	AATGTAAGAG	AAATTAAATC	TGAATAAAGA	2100
2101	ATTCTTCCTG	TTCACTGGCT	CTTTCTTCT	CCATGCACTG	AGCATCTGCT	TTTTGGAAGG	2160
2161	CCCTGGGTTA	GTAGTGGAGA	TGCTAAGGTA	AGCCAGACTC	ATACCCACCC	ATAGGGTCGT	2220
2221	AGAGTCTAGG	AGCTGCAGTC	ACGTAATCGA	GGTGGCAAGA	TGTCCTCTAA	AGATGTAGGG	2280
2281	AAAAGTGAGA	GAGGGGTGAG	GGTGTGGGGC	TCCGGGTGAG	AGTGGTGGAG	TGTCAATGCC	2340
2341	CTGAGCTGGG	GCATTTTGGG	CTTTGGGAAA	CTGCAGTTCC	TTCTGGGGGA	GCTGATTGTA	2400
2401	ATGATCTTGG	GTGGATCC					2418
	10	20	30	40	50	60	

23. Method for treating a patient with a tumor, comprising:

(i) identifying a tumor rejection antigen expressed by the tumor;

(ii) identifying a cell line which expresses an immunologically identical tumor rejection antigen;

(iii) treating a sample of said cell line to render it non-replicable, and

(iv) administering an amount of said non-replicable cell line to said patient sufficient to generate a cytolytic T cell response which lyses cells of said tumor.

24. Method of claim 23, wherein said tumor is a melanoma.

25. Vaccine useful in treating a patient with a tumor comprising an amount of a non-replicable cell line which expresses a tumor rejection antigen immunologically identical to a tumor rejection antigen expressed by said tumor sufficient to provoke a cytotoxic T cell response leading to lysis of cells of said tumor.

26. Method for identifying a cytotoxic T cell useful in treating a patient with a tumor, comprising:

(i) identifying a tumor rejection antigen expressed by said tumor,

(ii) contacting a cell expressing said antigen to a cytotoxic T cell, and



(iii) measuring tumor necrosis factor (TF) released by said cytotoxic T cell, wherein release of TNF is indicative of a cytotoxic T cell against said tumor.